VITROS Products Chemistry

INSTRUCTIONS FOR USE

ALT

VITROS Chemistry Products ALT Slides

Alanine Aminotransferase

REF **153 7513**
165 5281

Intended Use

For *in vitro* diagnostic use only.

VITROS ALT Slides quantitatively measure alanine aminotransferase (ALT) activity in serum and plasma.

Summary and Explanation of the Test

Alanine aminotransferase is present in high activity in liver, skeletal muscle, heart, and kidney. Serum ALT increases rapidly in liver cell necrosis, hepatitis, hepatic cirrhosis, liver tumors, obstructive jaundice, Reye's syndrome, extensive trauma to skeletal muscle, myositis, myocarditis, and myocardial infarction. [1]

Principles of the Procedure

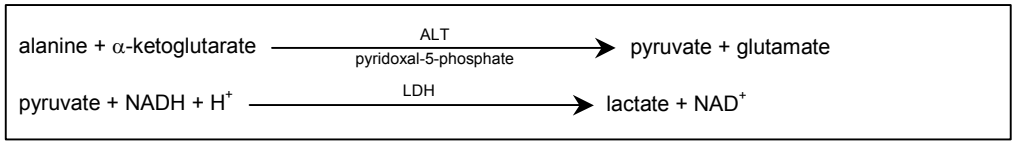
The VITROS ALT Slide method is performed using the VITROS ALT Slides and the VITROS Chemistry Products Calibrator Kit 3 on VITROS Chemistry Systems.

The VITROS ALT Slide is a multilayered, analytical element coated on a polyester support.

A drop of patient sample is deposited on the slide and is evenly distributed by the spreading layer to the underlying layers. The spreading layer contains the ALT substrates L-alanine and sodium α-ketoglutarate. Alanine aminotransferase catalyzes the transfer of the amino group of L-alanine to α-ketoglutarate to produce pyruvate and glutamate. Lactate dehydrogenase (LDH) then catalyzes the conversion of pyruvate and NADH to lactate and NAD^+.

The rate of oxidation of NADH is monitored by reflectance spectrophotometry. The rate of change in reflection density is proportional to enzyme activity.

Reaction Sequence



Test Type and Conditions

Test Type and Conditions for ALT

Test Type	VITROS System	Approximate Incubation Time	Temperature	Wavelength	Sample Drop Volume
Multiple-point rate	5,1 FS, 950, 750, 550, 250	5 minutes	37°C (98.6°F)	340 nm	11 µL

Warnings and Precautions

For *in vitro* diagnostic use only.

Take care when handling materials and samples of human origin. Since no test method can offer complete assurance that infectious agents are absent, consider all clinical specimens, controls, and calibrators potentially infectious. Handle specimens, solid and liquid waste, and test components in accordance with local regulations and NCCLS Guideline M29 [2] or other published biohazard safety guidelines.

For specific warnings and precautions for calibrators, quality control materials, and other components, refer to the Instructions for Use for the appropriate VITROS product, or to other manufacturer's product literature.

Reagents

Slide Ingredients

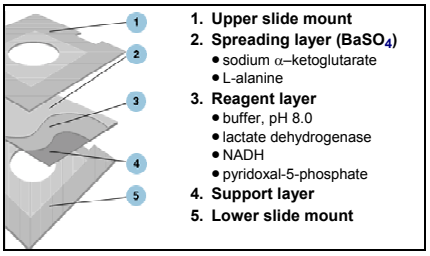
Reactive ingredients per cm²

Lactate dehydrogenase (porcine muscle, E.C.1.1.1.27) 0.12 U; L-alanine 0.86 mg; sodium α-ketoglutarate 54 µg; nicotinamide adenine dinucleotide, reduced 35 µg; and sodium pyridoxal-5-phosphate 11 µg.

Other ingredients

Pigment, binders, buffer, surfactants, cross-linking agent and stabilizer.

Slide Diagram



Cartridge Handling

CAUTION: **Do not use slide cartridges with damaged or incompletely sealed packaging.**

- Inspect the packaging for signs of damage.
- Be careful when opening the outer packaging with a sharp instrument so as to avoid damage to the individual product packaging.

Cartridge Preparation

IMPORTANT: *The slide cartridge must reach room temperature, 18°–28°C (64°–82°F), before it is unwrapped and loaded into the slide supply.*

1. Remove the slide cartridges from storage.
2. Warm the wrapped cartridge at room temperature for 30 minutes when taken from the refrigerator or 60 minutes from the freezer.
3. Unwrap and load the cartridge into the slide supply.

NOTE: Load the cartridges within 24 hours after they reach room temperature, 18°–28°C (64°–82°F).

Slide Storage and Stability

VITROS ALT Slides are stable until the expiration date on the carton when they are stored and handled as specified.

Slide Storage and Stability for ALT

Slide Cartridges	Storage Condition		Stability
Unopened	Refrigerated	2°–8°C (36°–46°F)	Until expiration date
	Frozen	≤-18°C (≤0°F)	Until expiration date
Opened	On-analyzer	System turned on	≤4 weeks
	On-analyzer	System turned off	≤2 hours

- Verify performance with quality control materials:
  - If the system is turned off for more than 2 hours.
  - After reloading cartridges that have been removed from the slide supply and stored for later use.

Specimen Requirements

WARNING: ***Handle specimens as biohazardous material.***

Specimens Recommended

- Serum
- Plasma: EDTA
  Heparin

IMPORTANT: *Certain collection devices have been reported to affect other analytes and tests.*[3] *Confirm that your collection devices are compatible with this test.*

Specimens Not Recommended

- Do not use hemolyzed specimens.[4]

Serum and Plasma

Specimen Collection and Preparation

Collect specimens using standard laboratory procedures.[5, 6]

NOTE: For details on minimum fill volume requirements, refer to the operating instructions for your VITROS Chemistry System.

Patient Preparation

- No special patient preparation is necessary.

Special Precautions

- Centrifuge specimens and remove the serum or plasma from the cellular material within 3 days of collection.[7]

Specimen Handling and Storage

WARNING: ***Handle specimens as biohazardous material.***

- Handle and store specimens in stoppered containers to avoid contamination and evaporation.
- Mix samples by gentle inversion and bring to room temperature, 18°–28°C (64°–82°F), prior to analysis.

IMPORTANT: *Do not freeze the specimen.*

Specimen Storage and Stability for ALT: Serum and Plasma[7]

Storage	Temperature	Stability
Room temperature	18°–28°C (64°–82°F)	≤3 days
Refrigerated	2°–8°C (36°–46°F)	≤1 week
Frozen	≤-18°C (≤0°F)	Not recommended

Testing Procedure

Materials Provided

- VITROS Chemistry Products ALT Slides

Materials Required But Not Provided

- VITROS Chemistry Products Calibrator Kit 3
- Quality control materials, such as VITROS Chemistry Products Performance Verifier I and II
- VITROS Chemistry Products 7% BSA
- VITROS Chemistry Products FS Diluent Pack 2 (BSA/Saline) (for on-analyzer dilution)

Operating Instructions

- Check reagent inventories at least daily to ensure that quantities are sufficient for the planned workload.
- For additional information, refer to the operating instructions for your VITROS Chemistry System.

IMPORTANT: *Bring all fluids and samples to room temperature, 18°–28°C (64°–82°F), prior to analysis.*

Sample Dilution

Serum and Plasma

If alanine aminotransferase activities exceed the system's reportable (dynamic) range:

Manual Sample Dilution

1. Dilute the sample with VITROS 7% BSA.
2. Reanalyze.
3. Multiply the results by the dilution factor to obtain an estimate of the original sample's alanine aminotransferase activity.

On-Analyzer Sample Dilution (VITROS 5,1 FS and VITROS 250 only)

Refer to the VITROS Chemistry System operating instructions for more information on the On-Analyzer Dilution Procedure. For VITROS 5,1 FS, use VITROS Chemistry Products FS Diluent Pack 2 for the dilution.

Calibration

Required Calibrators

VITROS Chemistry Products Calibrator Kit 3

Calibrator Preparation, Handling, and Storage

Refer to the Instructions for Use for VITROS Calibrator Kit 3.

Calibration Procedure

Refer to the operating instructions for your VITROS Chemistry System.

When to Calibrate

Calibrate:

- When the slide lot number changes.
- When critical system parts are replaced due to service or maintenance.
- When government regulations require.
  - For example, in the USA, CLIA regulations require calibration or calibration verification at least once every six months.

The VITROS ALT test may also need to be calibrated:

- If quality control results are consistently outside acceptable range.
- After certain service procedures have been performed.

For additional information, refer to the operating instructions for your VITROS Chemistry System.

Calculations

Based on sequential readings of the slide's reflectance at 340 nm over the defined incubation period, a rate of change in reflectance is determined. This rate is used in the software-resident multi-point rate calibration model to compute enzyme activity. Once a calibration has been performed for each slide lot, alanine aminotransferase activity in unknown samples can be determined from the rate of change in reflectance measured for each unknown test slide.

Validity of a Calibration

Calibration parameters are automatically assessed by the VITROS Chemistry System against a set of quality parameters detailed in the Coefficients and Limits screen (for VITROS 5,1 FS, see the Review Assay Data screen). Failure to meet any of the pre-defined quality parameters results in a failed calibration. The calibration report should be used in conjunction with quality control results to determine the validity of a calibration.

Reportable (Dynamic) Range

Reportable (Dynamic) Range for ALT

Conventional and SI Units (U/L)	Alternate Units (µkat/L)
3–1000	0.05–16.70

For out-of-range samples, refer to "Sample Dilution."

Traceability of the Calibration

Values assigned to the VITROS Chemistry Products Calibrator Kit 3 for alanine aminotransferase are traceable to the alanine aminotransferase method recommended by the International Federation of Clinical Chemistry (IFCC),[8] adapted to a centrifugal analyzer at 37°C.

Quality Control

Procedure Recommendations

WARNING: ***Handle quality control materials as biohazardous material.***

- Choose control levels that check the clinically relevant range.
- Analyze quality control materials in the same manner as patient samples, before or during patient sample processing.
- To verify system performance, analyze control materials:
  - After calibration.
  - According to local regulations or at least once each day that the test is being performed.
  - After specified service procedures are performed. Refer to the operating instructions for your VITROS Chemistry System.
- If control results fall outside your acceptable range, investigate the cause before deciding whether to report patient results.

- For general quality control recommendations, refer *to Statistical Quality Control for Quantitative Measurements: Principles and Definitions; Approved Guideline-Second Edition*[9] or other published guidelines.
- For additional information, refer to the operating instructions for your VITROS Chemistry System.

Quality Control Material Selection

IMPORTANT: *VITROS Performance Verifiers are recommended for use with the VITROS Chemistry System. Evaluate the performance of other commercial control fluids for compatibility with this test before using for quality control.*

- Control materials other than VITROS Performance Verifiers may show a difference when compared with other alanine aminotransferase methods if they:
  - Depart from a true human matrix.
  - Contain high concentrations of preservatives, stabilizers, or other nonphysiological additives.
- Enzyme activity might also vary with enzyme source, diluent temperature, and activation time during reconstitution.
- Do not use control materials stabilized with ethylene glycol.

Quality Control Material Preparation and Storage

Refer to the Instructions for Use for VITROS Chemistry Products Performance Verifier I and II or to other manufacturer's product literature.

Expected Values and Reporting Units

Reference Interval

These reference intervals are the central 95% of results from an internal study of 2444 apparently healthy adults (547 females and 1897 males).

Reference Interval for ALT

	Conventional and SI Units (U/L)	Alternate Units (µkat/L)
Adult	13–69	0.2–1.2
Females	9–52	0.2–0.9
Males	21–72	0.4–1.2

Each laboratory should confirm the validity of these intervals for the population it serves.

Reporting Units and Unit Conversion

The VITROS Chemistry System may be programmed to report ALT results in conventional, SI, and alternate units.

Reporting Units and Unit Conversion for ALT

Conventional/SI Units	Alt Units
U/L	µkat/L (U/L x 0.0167)

Limitations of the Procedure

Known Interferences

None identified.

Other Limitations

Certain drugs and clinical conditions are known to alter alanine aminotransferase activity *in vivo*. For additional information, refer to one of the published summaries. [10, 11]

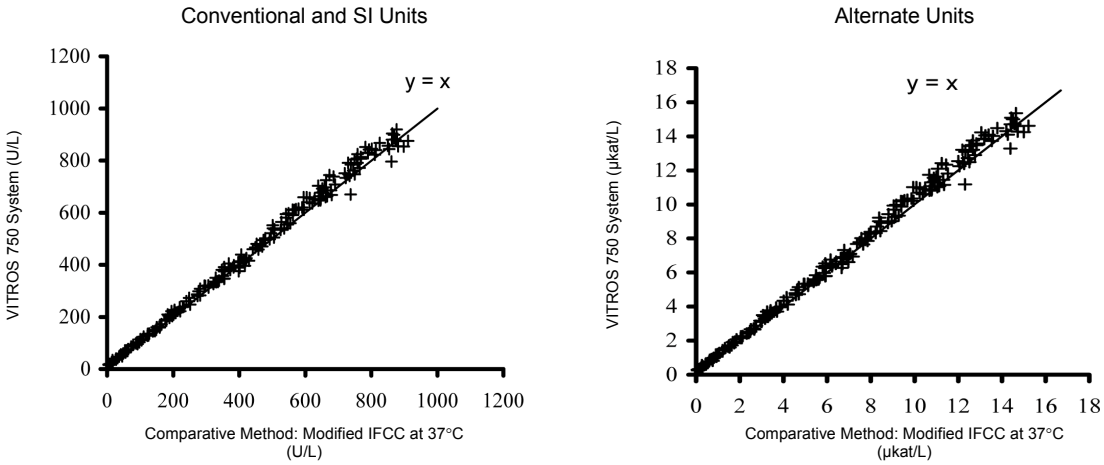
Performance Characteristics

Method Comparison

The plot and table show the results of a comparison of samples analyzed on the VITROS 750 System with those analyzed using the IFCC comparative method,[8] adapted to a centrifugal analyzer at 37°C. Testing followed NCCLS Protocol EP9.[12]

The table also shows the results of comparisons of the VITROS 250 and 950 Systems with the VITROS 750 System, and comparisons of the 5,1 FS System with the 950 System.

Method Comparison for ALT: Serum



Method Comparison for ALT: Serum

				Conventional and SI Units (U/L)			Alternate Units (µkat/L)		
	n	Slope	Correlation Coefficient	Range of Sample Activity	Intercept	Sy.x	Range of Sample Activity	Intercept	Sy.x
750 System vs. comparative method	195	1.02	0.997	4–911	+9.2	20.3	0.1–15.2	+0.15	0.34
250 System vs. 750 System	60	1.01	0.999	14–373	-0.6	2.6	0.2–6.2	-0.01	0.04
950 System vs. 750 System	122	0.99	0.999	9–988	-0.9	2.4	0.2–16.5	-0.02	0.04
5,1 FS System vs. 950 System	134	1.00	1.000	11–886	+1.7	2.3	0.2–14.8	+0.03	0.04

Precision

Precision was evaluated with quality control materials on VITROS 250, 750, 950, and 5,1 FS Systems following NCCLS Protocol EP5.[13]

The data presented are a representation of test performance and are provided as a guideline. Variables such as sample handling and storage, reagent handling and storage, laboratory environment, and system maintenance can affect reproducibility of test results.

Precision for ALT: Serum

System	Conventional and SI Units (U/L)			Alternate Units (µkat/L)			Within Lab CV%**	No. Observ.	No. Days
	Mean Activity	Within Day SD*	Within Lab SD**	Mean Activity	Within Day SD*	Within Lab SD**			
VITROS 250	37	3.5	4.1	0.6	0.06	0.07	11.2	78	20
	204	3.8	4.9	3.4	0.06	0.08	2.4	78	20
VITROS 750	34	2.0	2.7	0.6	0.03	0.05	8.1	90	23
	189	2.3	3.7	3.2	0.04	0.06	2.0	92	23
VITROS 950	34	1.7	3.2	0.6	0.03	0.05	9.6	84	23
	186	1.7	3.2	3.1	0.03	0.05	1.7	86	23
VITROS 5,1 FS	44	1.6	2.9	0.7	0.03	0.05	6.6	96	22
	187	1.9	3.5	3.1	0.03	0.06	1.9	96	22

* Within Day precision was determined using two runs/day with two to three replications.

** Within Lab precision was determined using a single lot of slides and calibrating weekly.

Specificity

Substances That Do Not Interfere

The substances listed in the table were tested with VITROS ALT Slides following NCCLS Protocol EP7 [14] and found not to interfere, bias <5.5 U/L, at the concentration shown.

Substances That Do Not Interfere With ALT

Compound	Concentration	
AST	200 U/L	200 U/L
Ascorbic Acid	3 mg/dL	170 µmol/L
Bilirubin	40 mg/dL	684 µmol/L
Ethanol	300 mg/dL	65 mmol/L

Compound	Concentration	
Glutathione	1 mg/dL	33 µmol/L
Intralipid	800 mg/dL	8 g/L
Salicylic acid	35 mg/dL	2.5 mmol/L

References

1. Tietz NW (ed). *Fundamentals of Clinical Chemistry*. ed. 3. Philadelphia: WB Saunders; 369–371; 1987.
2. NCCLS. *Protection of Laboratory Workers from Instrument Biohazards and Infectious Diseases Transmitted by Blood, Body Fluids and Tissue; Approved Guideline*. NCCLS Document M29 (ISBN 1-56238). NCCLS, Wayne, PA 19087; 1997.
3. Calam RR. Specimen Processing Separator Gels: An Update. *J Clin Immunoassay*. 11:86–90; 1988.
4. Young DS. *Effects of Preanalytical Variables on Clinical Laboratory Tests*. Washington D.C.: AACC Press; 3–7; 1993.
5. NCCLS. *Procedures for the Collection of Diagnostic Blood Specimens by Venipuncture*. NCCLS Document H3. Wayne, PA: NCCLS; 1991.
6. NCCLS. *Procedures for the Collection of Diagnostic Blood Specimens by Skin Puncture*. NCCLS Document H4. Wayne, PA: NCCLS; 1991.
7. *Clinical Laboratory Handbook for Patient Preparation and Specimen Handling*. Fascicle VI: Chemistry/Clinical Microscopy. Northfield, IL: College of American Pathologists; 1992.
8. Bergmeyer HU, Horder M, Rej R. Approved Recommendation (1985) on IFCC Methods for the Measurement of Catalytic Concentration of Enzymes. Part 3. IFCC Method for Alanine Aminotransferase. *J. Clin. Chem. Clin. Biochem.* 24:481; 1986.
9. NCCLS. *Statistical Quality Control for Quantitative Measurements: Principles and Definitions; Approved Guideline-Second Edition*. NCCLS Document C24. Wayne, PA: NCCLS; 1999.
10. Young DS. *Effects of Drugs on Clinical Laboratory Tests*. ed. 4. Washington D.C.: AACC Press; 1995.
11. Friedman RB, Young DS. *Effects of Disease on Clinical Laboratory Tests*. Washington, D.C.: AACC Press; 1990.
12. NCCLS. *Method Comparison and Bias Estimation Using Patient Samples; Approved Guideline*. NCCLS Document EP9. Wayne, PA: NCCLS; 1995.
13. NCCLS. *User Evaluation of Precision Performance with Clinical Chemistry Devices*. NCCLS Document EP5. Wayne, PA: NCCLS; 1992.
14. NCCLS. *Interference Testing in Clinical Chemistry*. NCCLS Document EP7. Wayne, PA: NCCLS; 1986.

Glossary of Symbols

The following symbols may have been used in the labeling of this product.

Do Not Reuse	Upper Limit of Temperature	This end up
Use by or Expiration Date (Year-Month-Day)	Lower Limit of Temperature	SI Units
LOT Lot Number	Temperature Limitation	CONV Conventional Units
SN Serial Number	Consult Instructions for Use	Value
REF Catalog Number or Product Code	Xi Irritant	Range
Attention: See Instructions for Use.	Xn Harmful	Range of Means
Manufacturer	T Toxic	Midpoint
EC REP Authorized Representative in the European Community	Fragile, Handle with Care.	Revised
Σ Contains Sufficient for "n" Tests	Keep Dry	Supersedes
IVD *In vitro* Diagnostic Medical Device	Der Grüne Punkt (the Green Dot). Manufacturer follows certain packaging material waste disposal management regulations.	σ Estimate within-lab SD.

Revision History

Date of Revision	Version	Description of Technical Changes*
2004-09-13	3.0	• Added VITROS 5,1 FS Chemistry System • Specificity – added Intralipid; updated Bilirubin • Glossary of Symbols – updated data
2003-07-28	2.0	• New organization and sections consistent with IVD Directive • Reference Interval – adult: corrected values • Limitations of the Procedure – removed statements regarding elevated total protein • Method Comparison – updated comparisons for 750 System and plots • Precision – updated data for the 750 System • References – added 2, 3, 12, 13, 14
2002APR19	1.0 – English only	New format, technically equivalent to 11/96.

* The change bars indicate the position of a technical amendment to the text with respect to the previous version of the document.

When this Instructions For Use is replaced, sign and date below and retain as specified by local regulations or laboratory policies, as appropriate.

_____ _____

Signature Obsolete Date

CE

EC REP

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